CURRENT TOPICS

The pharmacology of inhaled nitric oxide

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In 1980 Furchgott and Zawadski reported that endothelial cells stimulated by acetylcholine produced a vasodilator substance which relaxed vascular smooth muscle.[1] The identity of this endothelium derived relaxing factor (EDRF) remained elusive until 1987 when Palmer and colleagues showed that the effects of nitric oxide (NO) accounted for the known biological actions of EDRF,[2] and that endothelial cells synthesised NO from L-arginine and oxygen.[3]

The demonstration that analogues of L-arginine, such as N[G]-monomethyl-L-arginine (L-NMMA) and N[G]-nitro-L-arginine methyl ester (L-NAME), prevented the production of NO by cells provided an experimental tool for examining the widespread biological roles of NO.[4] Administration of L-NMMA or L-NAME to animals blocked the vasodilator action of acetylcholine, caused a persistent rise in blood pressure, and reduced the blood volume of organs such as the brain, demonstrating both that NO release contributes to a constant vasodilator tone, and that further release can be initiated by appropriate stimuli.[5 6] Conversely, administration of drugs which produced NO, such as the nitrate vasodilators, produced a fall in blood pressure.[5]

Several isoforms of the enzyme nitric oxide synthase (NOS) have been characterised – both constitutive and inducible. Constitutive enzymes were found in many tissues, including endothelium and brain.[5 7] An inducible form of the enzyme, which was expressed after cytokine stimulation and during sepsis, was found in macrophages.[5 8] NO is now known to be a multifunctional biological mediator with diverse roles in the cardiovascular, neurological, immunological and many other systems.[9]

Agonists and antagonists of NO have many potential medicinal roles, but among the first to be examined in detail is the use of inhaled NO as a specific pulmonary vasodilator. Information about this novel drug is accumulating, although there remain extensive gaps in our knowledge of its pharmacology and toxicology.

Chemistry

Nitric oxide (nitrogen monoxide) is a volatile gas which is the second (nitrogen oxidation state +2) in the sequence of oxy-compounds of nitrogen that includes NO⁻ (oxidation state +1) and NO⁺ (oxidation state +3). The characteristic chemistry of NO is largely due to the arrangement of the electrons in the valence shell. Nitrogen and oxygen combine to form four bonding and four antibonding orbitals which are filled by 11 electrons, giving the molecule eight bonding and three antibonding electrons, a bond order of 2·5, and a bond length intermediate between double and triple bonding (1·150 A).[10]

The single unpaired electron in the 2*pπ molecular orbital defines the molecule as a free radical (the presence of this unpaired electron is sometimes emphasised by writing the formula as ·NO[10]). However, NO does not exhibit the high reactivity characteristic of most free radicals and shows little tendency to dimerise. Nevertheless, in the presence of superoxide radical reactions occur which lead to highly reactive species being formed[11]; these are described further below.

NO is thermodynamically unstable ($\Delta G^O_{formation}$=86.32 kJ), but decomposition is kinetically hindered and the gas and can be stored indefinitely at room temperature and 1 atmosphere pressure. At increased pressure, disproportionation of NO to N_2O and NO_2 can occur. This reaction obeys third order kinetics and at 30°C and 200 atmospheres pressure, some NO initially present may be converted into N_2O and NO_2. This needs to be considered when NO is stored in pressurised cylinders for clinical use.[10]

In aqueous solutions NO exhibits solubility and diffusibility similar to other diatomic gases. It does not interact with water, and this property is probably relevant to its pharmacological and biological effects. The diffusion coefficient of NO in tissue and the lipid membrane permeability have not yet been formally quantified.[11] NO is not affected by light.

The unpaired electron is antibonding and easily detached to allow NO⁺ to be formed. Although the lifetime of NO⁺ in aqueous media is very short, it has been suggested that the presence in tissue of alternative redox forms of NO might lead to potent changes in biological effects.[12]

Metabolism

Both the effects and metabolism of inhaled NO take place in the lung, where several of the chemical reactions of NO are relevant:

REACTIONS WITH HAEM CONTAINING METALLOPROTEINS

Reactions with haem containing proteins are rapid. The association rate constant with Fe(II) in haemoglobin is about 300 times greater than for oxygen. This reaction leads to the formation of Fe(III) haemoglobin (methaemoglobin) and NO_2^-. NO also binds to Fe(III) complexes and Fe(III)NO undergoes a transfer reaction to form Fe(II)NO$^+$. NO$^+$ can be oxidised to NO_2^- and NO_3^- so that NO is cleared rapidly from the blood.[11] The reaction of NO with haem groups is thought to be the predominant interaction in biological systems, and to account both for its activity in smooth muscle cells and its rapid inactivation in blood.[11]

FREE RADICAL REACTIONS

NO reacts with the superoxide anion (O_2^-), leading to the production of peroxynitrite ($OONO^-$). At physiological pH, $OONO^-$ has a half life of 1·9 seconds, being protonated to form ONOOH which undergoes spontaneous homolysis to form the highly reactive hydroxyl radical ($\cdot OH^-$).[13] The hydroxyl radical is highly damaging to lipid membranes, and consequently the potential for NO administration to induce tissue injury has led to a great deal of interest in this reaction, and to the role of NO as a cell toxin.[14]

AUTOXIDATION

The reaction between NO and O_2 to form NO_2 is rapid at high concentrations of NO, but slow at the concentrations used in trials of inhaled NO treatment. In air, for low concentrations of NO the half life of NO and the rate of NO_2 formation depend on the initial concentration; the half life may vary between one and 500 seconds.[10] It is reported that at NO concentrations of 40 parts per million (ppm) or less, combination of NO with pure oxygen requires 2·56 minutes to yield 5 ppm NO_2, although at 120 ppm NO this time falls to 0·26 minutes.[15] NO_2 is an environmental toxin which can cause pulmonary oedema, haemorrhage, and bronchiolitis obliterans,[16] and inhalation of NO_2 at concentrations as low as 2 ppm caused lung epithelial damage. A considerable body of published data exists on the effects of inhaled NO_2, suggesting that 5 ppm is a maximum safe dose.[17] Clinical administration systems should minimise the period of NO and O_2 mixing to prevent extracorporeal NO_2 formation. Within tissue NO has a higher affinity for haemoglobin than oxygen, minimising autoxidation in blood.

FORMATION OF NITROSOTHIOL COMPOUNDS

NO undergoes nitrosation reactions, and in biological systems the formation of nitrosothiols seems to be favoured. The reaction of NO with RSH to form RSNO is rapid but the resulting nitrosothiol is unstable and the reaction is reversible.[10] The presence of nitrosothiols in human plasma supports the suggestion that nitrosothiols may represent a 'stabilised' form of NO in biological tissue.[18]

Pharmacokinetics

Inhaled NO is taken up into the precapillary airspaces and alveoli at a rate many times faster than inhaled oxygen, and the diffusing capacity of the lung is 4·5 times higher for NO than for carbon monoxide.[19] The anatomical proximity of the airspaces to muscular arterioles allows NO to diffuse into contact with the abluminal surface of these vessels.[19]

Because of rapid removal of NO by reaction with haemoglobin, the effects of NO inhalation are generally considered to be localised to lung tissue with an effective half life of 2–6 seconds.[20] The half life of potential 'stabilised' forms of NO (S-nitrosothiols) is longer, and nitrosothiols may prolong the actions of NO in tissue.[9][21] The metabolites of NO are cleared from the body by the kidneys within five to eight hours[9] and concentrations of methaemoglobin rarely rise above 1–2% during NO treatment.[22][23]

Pharmacology

MECHANISMS OF ACTION

Inhaled NO diffuses from alveoli into cells[16] where it binds to the prosthetic haem group of the soluble guanylate cyclase enzyme (GTP pyrophosphatelyase (cyclising); EC 4.6.1.2).[24] NO binding leads to a conformational change similar the effect of oxygen,[25] and causes a rise in intracellular cyclic guanylate monophosphate (cGMP), which in turn leads to muscular relaxation and vasodilation (figure). An independent effect of NO on calcium dependent potassium channels has also been described,[26] and preliminary evidence suggests a direct activation of G-proteins.[27] NO may also stimulate prostanoid synthesis by reacting with the haem in prostaglandin H synthase.[28]

It remains controversial whether these effects are entirely due to the action of NO itself, or also to nitrosothiol metabolites such
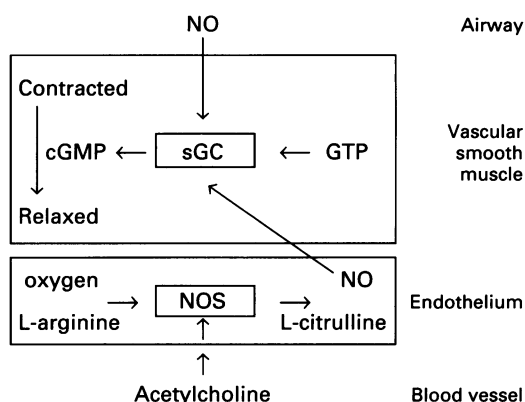
Physiological and pharmacological actions of NO on vascular smooth muscle. Physiological stimuli, such as acetylcholine, activate constitutive nitric oxide synthase (NOS) in endothelial cells, which converts L-arginine and oxygen to L-citrulline and nitric oxide (NO). NO diffuses to smooth muscle cells and activates soluble guanylate cyclase (cGC) which converts GTP to cyclic-GMP, leading to muscular relaxation. Pharmacological administration of NO into the airways acts directly on guanylate cyclase.

as S-nitrosocysteine.[29] [30] Inhaled NO may also have some distant effects mediated by nitrosothiols, as prolongation of the bleeding time has been reported in healthy adult volunteers who inhaled NO,[31] and rats treated with inhaled NO developed prolonged brainstem evoked responses and impaired learning.[32] However, such actions are probably minor.

PHARMACOLOGICAL EFFECTS

NO mediated relaxation of vascular smooth muscle leads to vasodilation[5] and bronchodilation.[16] [33] In newborn lambs and piglets it has no effect on the normal pulmonary vasculature, but reverses the vascoconstriction induced by hypoxia or vasoconstrictor prostanoids, and this effect is not abolished by concomitant acidaemia.[34–36] Vasodilation can occur in the presence of endothelial damage which abolishes the vasodilatory effect of acetylcholine,[37] and it is more effective than inhalation of prostaglandin I_2.[38] In piglets inhaled NO reduces the vasoconstriction associated with bacterial sepsis.[39]

Because inhaled NO is delivered to ventilated lung segments, its vasodilatory effect improves ventilation:perfusion matching and oxygen transport so that improved oxygenation has been observed even without a fall in pulmonary artery pressure.[40] This effect may be potentiated by the effects of NO on bronchial smooth muscle: inhaled NO reduces the bronchconstrictor effect of inhaled metacholine in guinea pigs and rabbits, although at inhaled concentrations of 80–300 ppm, higher than those required for vasodilation (5–80 ppm).[16]

Other actions of NO may be relevant during inhalation treatment: leakage of albumin into the alveolar space after pulmonary injury is reduced[41]; adhesion and aggregation of platelets and leucocytes are inhibited[5] [42]; the effects of endothelin-1 and other vasoactive agents on pulmonary vessels may be antagonised.[43] [44] NO has a negative feedback effect on NO synthesis, raising concern that inhaled NO may depress endogenous pulmonary vasodilatation.[45–47]

NO has many further biological effects, including inhibition of platelet aggregation[5] and a negative inotropic effect.[48] Cytokine activated macrophages use NO to kill tumour cells, fungi, bacteria, viruses and helminths.[8] NO can disrupt enzymes in both the Krebs cycle (aconitase), mitochondrial complex I (reduced nicotinamide-adenine dinucleotide phosphate-NADH dehydrogenase), and mitochondrial complex II (succinate-NADH dehydrogenase), as well as the rate limiting enzyme in DNA synthesis (ribonucleoside-diphospate reductase).[49] NO modulates gene transcription and post-transcription processing[50] and may play an important part in inflammation: it enhances the effects of cyclo-oxygenase I and II and stimulates the production of some proinflammatory eiconosoids.[28] Immune complex induced injury in rat lung[51] and inflammatory damage to other tissues are reduced by NO inhibitors.[52] [53] NO acts as a

neurotransmitter in the central and peripheral nervous systems, notably in non-adrenergic, non-cholinergic fibres, which are prominent in lung.[16] NO has been implicated as a mediator of cell death in the nervous system.[54]

Toxicology

Inhalational NO is not a licensed drug, and full toxicology data are unavailable. Although the many functions of NO in the body mean that modulation of NO metabolism or effects could have far reaching results, it is generally assumed that systemic toxicity is low because of rapid inactivation of NO on contact with blood.

Studies in rats have shown that inhalation of up to 1500 ppm NO for 15 minutes caused no demonstrable lung injury.[55] Overdose of 20 000 ppm caused acute pulmonary oedema and methaemoglobinaemia.[16] Unfortunately, such studies are of limited relevance to clinical uses of the drug.

Reports of adverse effects in clinical studies have been rare: paradoxical hypoxia during NO inhalation in one newborn infant has been reported, and it was suggested (without supporting evidence) that this was related to maternal indomethacin treatment.[56] The increase in bleeding time demonstrated in adult volunteers does not seem to be a significant problem in clinical practice.[31]

Nevertheless, there are theoretical reasons for caution in the use of inhaled NO treatment. The formation of hydroxyl radicals might cause oxidative damage to tissue.[14] NO might impair energy metabolism[57] or promote inflammation,[28] and it has been suggested that NO is involved in mediating the effects of pertussis toxin in airways.[58] DNA damage in *Salmonella* species due to NO has been recorded, and as cigarette smoke contains NO this has been proposed as one mechanism for the carcinogenic effect of smoking.[59] [60] Some evidence of deteriorations in lung function during inhalation of NO has been produced in studies of adult volunteers: a mean fall in arterial oxygen tension of 7 mm Hg was recorded in 191 normal subjects inhaling NO of 15–20 ppm, and at higher concentrations airways resistance increased.[16]

Conclusion

NO is a multifunctional biological mediator with clear pharmacological effects when inhaled into the lung. Considerable gaps remain in our understanding of the pharmacology and toxicology of this novel inhalational treatment, and enthusiasm for NO treatment should be tempered with caution.

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